OxyScan™ SOD-525 Assay System

Automated Colorimetric Determination of Superoxide Dismutase

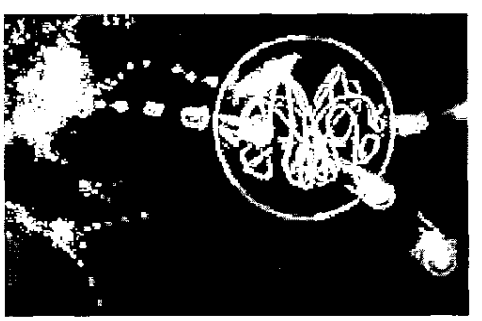
For Research Use Only. Not For Use in Diagnostic Procedures.
Catalog Number 51010
READ ENTIRE INSERT BEFORE BEGINNING ASSAYS!

SUMMARY AND EXPLANATION OF THE TEST ANALYTE

Superoxide dismutases (SOD) are metalloenzymes that catalyze the dismutation of superoxide anion into oxygen and hydrogen peroxide according to the following reaction:

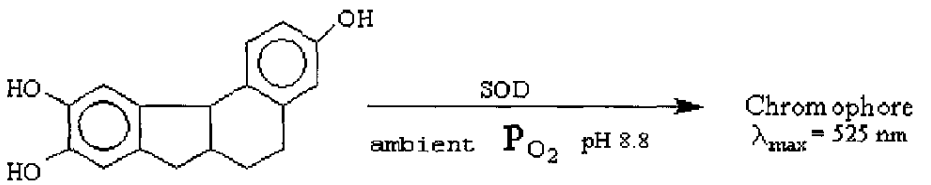
$$2O_2^{\cdot -} + 2H^+ \xrightarrow{SOD} H_2O_2 + O_2$$

Different types of SOD have been described. The three most studied types are characterized by the redox-active metals at the catalytic site. Cu/Zn-SOD is found primarily in eukaryotes, localized mainly in the cytosol and nucleus, as a dimer of identical 16,000 MW subunits. Fe-SOD, a dimer of identical 20,000 MW subunits, is present mainly in prokaryotes. Mn-SOD crosses the range from prokaryotes to eukaryotes, most abundantly in the mitochondria, and has been isolated as both dimers and tetramers of 21,000 MW identical subunits.[1] It is widely recognized that such enzymes provide a defense system that is essential for the survival of aerobic organisms.[2]

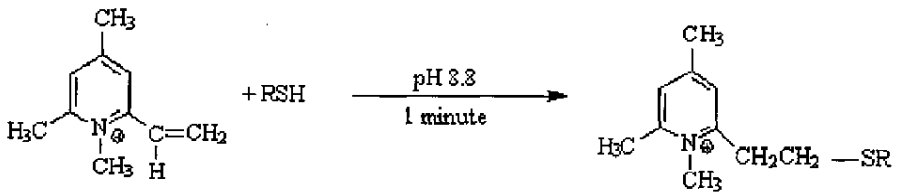


PRINCIPLES OF THE PROCEDURE

The OxyScan™ SOD-525 Assay measures the activity of all types of SOD. The method is based on the SOD-mediated increase in the rate of autoxidation of 5,6,6a,11b-tetrahydro-3,9,10-trihydroxybenzo[c]fluorene (**R1**) in aqueous alkaline solution to yield a chromophore with maximum absorbance at 525 nm. The chromophore has not been isolated or characterized.



Interference due to mercaptans (RSH), such as reduced glutathione, is controlled by pretreating samples with 1,4,6-trimethyl-2-vinylpyridinium **(R2)**, which directly eliminates mercaptans by means of a fast alkylation reaction.



REAGENTS

The OxyScan™ **SOD-525** reagent set is sufficient for 300 SOD determinations and consists of the following reagents:

R1	5,6,6a,11b-tetrahydro-3,9,10-trihydroxybenzo[c]fluorene, 0.094 mM, in 32 mM HCl containing 0.071 mM diethylenetriaminepentaacetic acid (DTPA) and 0.36% ethanol, 5 x 6.5 mL.
R2	1,4,6-trimethyl-2-vinylpyridinium trifluoromethanesulfonate, 4.8 mM, in 1 mM HCl, 5 x 6.5 mL.
Buffer	2-amino-2-methyl-1,3-propanediol, 112.5 mM, containing 7.425 mM boric acid and 0.2475 mM DTPA, pH = 9.4, each at 2 x 30 mL and 1 x 90 mL.

Reagent Preparation

OxyScan™ SOD-525 reagents are ready to use. The R1 and R2 reagents are supplied as single-use vials, and should be discarded after each session **OR** after 4 hours open in the reagent well. **DO NOT REPRIME** and **RE-USE** the R1 and R2 reagent vials.

Warnings and Precautions

For Research Use Only. Not For Use in Diagnostic Procedures.
For *In Vitro* Use Only.

Use established laboratory precautions when handling or disposing any chemicals contained in this product. Refer to the Material Safety Data Sheet for risk, hazard and safety information.

Reagent Storage and Stability

Store the reagents tightly closed at 2-8°C in the dark. **Do not freeze.** Unopened reagents are stable until expiration date printed on the label.

SAMPLE GUIDELINES

NOTE: The calibrators and controls must be treated with the same preparation procedures as the test samples, i.e., if the test samples are extracted (below), the calibrators and controls must also be extracted.
NOTE: Ethanol:chloroform extraction inactivates Mn-SOD and Fe-SOD, therefore, extracts will only contain Cu/Zn-SOD.

Potentially Interfering Substances

Compounds in the sample exhibiting redox properties may interfere with the SOD-525 method. Interference can be assessed by testing linearity of the measured activity upon sample dilution or by recovery of known amounts of SOD activity added to the sample. Interferents in the sample, other than the mercaptans removed with **R2**, may be removed by extraction, dialysis, or gel filtration.

Samples

- **Purified SOD** can be dissolved in water, saline, SOD Assay Buffer or other non-interfering buffer and assayed without further processing. If sample storage is necessary, it is recommended that samples be stored frozen at less than -60°C. Avoid freeze-thaw cycles.
- **Erythrocyte Lysates** are prepared by adding 4 volumes of ice-cold deionized water to the packed red blood cells (RBC). If sample storage is necessary, it is recommended that samples be stored frozen at less than -60°C. Avoid freeze-thaw cycles. Extraction of the RBC lysate with ethanol:chloroform (below) is required. Extracted lysates must be further diluted 1:4 in SOD Assay Buffer prior to assay. Retain a lysate sample for hemoglobin determination.
- **Mitochondria** containing Mn-SOD must be isolated by differential centrifugation prior to measuring SOD activity. The researcher is referred to Rice and Lindsay4 for detailed descriptions of methods for isolating mitochondria from various tissues.
- **Tissues** must be perfused or washed with saline containing 0.16 mg/mL heparin to remove RBC that will contribute to the SOD activity of the sample. Mince tissue in five parts cold buffer (0.25 M sucrose recommended), transfer the tissue to homogenizer (Potter-Elvehjem or Dounce) with an equal volume of cold buffer and homogenize. Centrifuge the homogenate at 8500 x g for 10 minutes at 4°C and collect the supernatant containing Cu/Zn-SOD. In general, tissue homogenates require extraction (below). If sample storage is necessary, it is recommended that samples be stored frozen at less than -60°C. Avoid freeze-thaw cycles. Retain a sample of the homogenate for protein determination.
- **Cultured Cells** can be harvested in 0.25 M sucrose, SOD Assay Buffer, or other non-interfering buffer. The cells are homogenized, or disrupted by several freeze-thaw cycles, and the homogenate is clarified by centrifugation at 8500 x g for 10 minutes at 4°C. Clarified cell culture supernatants can generally be assayed without extraction. If sample storage is necessary, it is recommended that samples be stored frozen at less than -60°C. Avoid freeze-thaw cycles. Retain a sample of the homogenate for protein determination. Approximately 2 x 106 cells or 0.5 - 1 mg protein per sample is required.

Extraction to Remove Interfering Substances

1. Prepare the Extraction Reagent with ethanol:chloroform 62.5:37.5 (v/v) and store at 2-8°C.
2. Add 400 µL of ice-cold Extraction Reagent to 250 µL of sample in a test tube.
3. Vortex for a minimum of 30 seconds and centrifuge at 3000 x g and 4°C for 5 minutes.
4. Collect the upper aqueous layer.
5. Dilute the extract 1:4 with assay buffer (e.g., 200 mL supernatant + 800 mL SOD buffer).
6. Place extract at 2-8°C if assayed within 24 hours; otherwise, store frozen at less

than -60°C.

ASSAY PROCEDURE

Instrument

OxyScan™ Instrument, Catalog Number 51101.

Materials Required But Not Provided

- OxyScan™ Cuvettes, Catalog Number 81003.
- OxyScan™ SOD-525™ Calibrator Kit, Catalog Number 52010.
- OxyScan™ 520 Photocalibrator, Catalog Number 51110.
- Pipettes with disposable tips.
- Fresh deionized water for Blank.

Perform Photocalibration

1. Follow instructions in OxyScan™ 520 nm Photocalibrator package insert.
2. Perform photocalibration at 520 nm.

Pump Preparation

1. Insert reagents into the proper wells:

R1	**Pump 1**
Empty	**Pump 2**
R2	**Pump 3**
Buffer	**Pump 4**

2. Insert proper tubing into the reagent bottles.
3. Lift the reagent arm and place the waste cup beneath the tubing exit.
4. Ensure the pump cover is in place.
5. Press PUMP ENGAGE/RELEASE to engage the pumps.
6. Press PRIME.
7. Select PRIME ALL.
8. When the screen changes to the step pumps, press each pump number to pipet 100 mL of fluid to confirm tubing is primed.
9. Press ESC (display does not change).

Instrument Preparation

1. Press UTILITY and scroll down to REPLICATE SELECTION.
2. Choose number of replicates (3 is recommended).
3. If running an assay calibration curve with the samples, perform steps 4 and 5.
4. Scroll down to the ASSAY CAL screen and choose the CONC.
5. Choose SOD and enter the calibrator concentration values from the Calibrator Kit package insert.

Cuvette/Carousel Preparation

1. Dilute all samples, calibrators and control 1:4 in SOD Assay Buffer.

2. Pipet 200 mL deionized water into the first two positions of the carousel to serve as instrument blanks.
3. If not running an assay calibration curve, proceed step 5.
4. Starting with position 3, pipet 50 mL of each diluted calibrator level into the appropriate number of replicates wells (Instrument Preparation step 2).
5. Pipet 50 mL of each diluted sample into the appropriate number of replicates wells.
6. Insert cuvette(s) into the carousel, starting with the first blank in position 1.
7. Close instrument cover.

Assay Run

1. Press ASSAYS and select YES or NO for assay calibration during run. Select SOD.
2. Enter the number of samples (Not total number of replicates, calibrators or blanks).
3. Press ENTER
4. (Optional) Press SAMPLE ID to enter numerical IDs for the samples including calibrators. Press ESC to exit the menu).
5. Ensure instrument cover is down.
6. Press RUN.

LIMITATIONS OF THE PROCEDURE

The SOD activity in plasma is below the detection limit of the SOD-525 assay. The utility of the Assay in plasma is limited and not recommended.

Cyanide interferes with the autoxidation of the chromogen (**R1**) and should be avoided. Cyanide cannot be used to distinguish Cu/Zn-SOD activity from Mn-SOD activity in samples.

The ethanol:chloroform Extraction Reagent inactivates Mn-SOD and Fe-SOD.

Whole tissue specimens should be free of trapped blood to prevent sample contamination by red cell SOD. The expected concentration of SOD in erythrocytes is much greater than found in most tissues. Rinse and/or perfuse tissue with 0.9% NaCl containing 0.16 mg/mL heparin.

BIBLIOGRAPHY

1. *Stallings, W., et al. The Three-Dimensional Structure of Iron Superoxide Dismutase:* Kinetics and Structural Comparisons with Cu/Zn and Mn Dismutases, Oxygen Radicals in Chemistry and Biology, Walter de Gruyter & Co., Berlin 779-792, 1984.
2. Beyer, W., et al. Superoxide Dismutases, Progress in Nucleic Acid Research and Molecular Biology, 40:221-253, 1991.
3. Nebot, C., et al. Spectrophotometric Assay of Superoxide Dismutase Activity Based on the Activated Autoxidation of a Tetracyclic Catechol, Analytical Biochemistry 214:442-451, 1993.
4. Rice, JE and Lindsay, JG (1997), Subcellular Fractionation of Mitochondria, Subcellular Fractionation A Practical Approach, (ed. J.M. Graham and D. Rickwood), p. 107. Oxford University Press Inc., New York.

SUMMARY

Catalog #	51010
Calibrator Requirement:	Also requires calibrator # 52010
Photo Calibrator Requirement:	Also requires OxyScan™ 520 Photocalibrator
Specimen Requirements:	SOD solutions, plasma, tissue homogenate, RBC lysate
Kit Contents:	(suitable for 100 triplicate determinations) Chromagenic Reagent Mercaptan Scavenger Buffer
Sensitivity:	0.2 U/mL SOD-525 enzyme activity.
Specificity:	SOD specific; Specific for Cu/Zn-SOD after ethanol/chloroform extraction

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